Studies on seed morphometry of *Habenaria* species from Western Ghats, India[a]

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Abstract

Seed morphometry of eighteen species of *Habenaria* was studied using light and scanning electron microscopy. These species showed remarkable variation in seed colour (off white, brown and yellow) and shape (fusiform, spathulate and filiform). Seven species were found to have elongate seeds, while the remaining species had truncate seeds. The number of testa cells was found to be more or less constant at the genus level, and with straight, sinuous, or undulate walls. Based on seed and embryo volumes, varied amounts of air space exist within the genus. Seeds with the highest percentage of air space were found in *H. gibsonii* (84.91 ± 11.34%) followed by *H. frucifera* (84.77 ± 9.45%) and *H. digitata* (81.56 ± 11.23%). These species show wide geographical distribution within India and abroad. On the other hand *H. grandifloriformis* which is endemic to India with restricted distribution shows the lowest percentage of air space (0.86 ± 0.46%). The present study shows that there is direct correlation between percent air space in the seeds, buoyancy of seed and distribution of species. Morphometric analysis of *Habenaria* seeds indicated that the length, width and number of testa cells as well as the presence/absence of reticulation on seed coat walls and the nature of this reticulation are of diagnostic value for delimitation of different species.

Résumé

Étude morphométrique des graines des espèces d'*Habenaria* des Western Ghats, Inde – La morphométrie des graines de dix-huit espèces d'*Habenaria* a été étudiée par microscopie optique et par microscopie électronique à balayage. Ces espèces

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ont montré une variabilité remarquable dans la couleur (blanc cassé, brun, jaune) et la forme (fusiforme, spatulée, filiforme) des graines. Chez sept espèces les graines sont allongées tandis que chez les autres elles sont tronquées. Le nombre de cellules de la testa s'est révélé plus ou moins constant au niveau du genre, les cellules ayant des parois droites, sinueuses ou ondulées. Sur la base des volumes de la graine et de l'embryon, la quantité d'air dans la graine s'est révélé variable au sein du genre. Les graines avec le plus fort pourcentage d'air ont été trouvées chez *H. gibsonii* (84.91 ± 11.34%), suivi par *H. frucifera* (84.77 ± 9.45%) *et H. digitata* (81.56 ± 11.23%). Ces espèces possèdent également une distribution géographique large en Inde et en dehors. A l'opposé, *H. grandifloriformis*, qui est endémique à l'Inde avec une distribution réduite, montre le plus faible pourcentage d'air (0.86 ± 0.46%). L'étude a montré qu'il existe une relation directe entre pourcentage d'air, flottabilité des graines et distribution des espèces. L'analyse morphométrique des graines d'*Habenaria* a indiqué que la longueur, la largeur et le nombre des cellules de la testa ainsi que la présence ou l'absence de réticulation sur la couche externe de la graine et la nature de cette réticulation sont des critères importants pour la délimitation des espèces.

Introduction

Orchidaceae are characterized by minute dusty seeds having oval, ellipsoidal to round endosperm enclosed within a thin transparent fusiform testa. This character reflects the advanced nature of the family and its developmental patterns. These seeds show a significant variation in their length and width which is of taxonomic importance at genus and species level (Arditti & Ghani, 2000). According to various workers (Clifford & Smith, 1969; Vij *et al.*, 1992; Rasmussen, 1995; Swamy *et al.*, 2004 and Verma *et al.*, 2013) the seed size in orchids has a direct correlation with plant habit: epiphytic orchids generally possess smaller seeds as compared to the terrestrials. However, the data presented by Arditti & Ghani (2000) on numerical and physical characteristics of orchid seeds suggest that this is not a rule. Molvray & Kores (1995) measured the size of various orchid seeds and ultimately concluded that, generally seed size ranges from 0.5 - 0.8 mm according to the genus taken into consideration. Arditti *et al.* (1979) and Vij *et al.* (1992) demonstrated that seeds show least shape variability, usually fusiform, in primitive orchids (Cypripedioideae) but exhibit great variations (fusiform, ovoid, elliptical, filamentous and cylindrical) in

advanced Epidendroid orchids. Fusiform seeds that are met in all of the subfamilies appear a primitive characteristic from which all other seed shapes might have evolved (Arditti *et al.*, 1979; 1980; Healey *et al.*, 1980; Rasmussen 1995; Verma *et al.*, 2013). According to Arditti *et al.* (1979) the relative degree of truncation of orchid seeds is directly correlated to an increase in their length rather than their width. They suggested that instead of length and width, the volume should be considered as a better measure of seed size in orchids. Furthermore Arditti *et al.*, (1980) and Augustine *et al.* (2001) suggested that the length/width ratio provides the information on relative degree of truncation of the seeds in orchids. Seeds having L/W < 6.0 are referred to as truncated seeds whereas seeds having L/W > 6.0 are referred to as elongated seeds. Arditti *et al.* (1980) and Barthlott and Ziegler (1981) studied various characters of seeds with the help of light and scanning electron microscopes and gave a detailed description of the structure and the types of seeds. The testa cells are quadrilateral in shape in most of the presently studied species. Earlier, Clifford & Smith (1969), Vij *et al.* (2006) and Verma *et al.* (2013) suggested that quadrilateral testa cells are found commonly in ground growing species, and they are generally fusiform in epiphytes. Kurzweil (1993) suggested that the seed coat in orchids may be made up of cells that differ in shape and ornamentation; cells are generally concave and elongated with straight or slightly undulate anticlinal walls. Arditti *et al.* (1979) proposed a relationship between volume and size of the seeds. According to them the seed volume and seed size are directly proportional to each other. Seeds with higher ratio "seed volume/embryo volume" are expected to be more buoyant and the corresponding species are more widely distributed than those with a lower ratio. According to Augustine *et al.* (2001) studies on seed morphometry provide very useful data in solving some problems of taxonomy, phylogeny and phytogeography. Most of the earlier workers focused and discussed the significance of the presence of air space in the seeds of orchids, which makes the seeds very light and buoyant. Even Augustine *et al.* (2001) suggested that very light and buoyant seeds with a greater percentage of air space may get dispersed over wide range of geographical areas, whereas the seeds with minimum air space show a more restricted and localized distribution. Arditti & Ghani (2000) suggested that the increase in the percentage of air space is due to an increase of the cell length of the testa.

The genus *Habenaria* is totally ignored or poorly studied for seed characters and seed morphometry as a taxonomical tool, except very few studies regarding the importance of seed morphometry. Hence in the present study an attempt is carried out to study in detail the various characters of seeds and its correlation with species distribution pattern within this neglected genus.

Material and Methods

Mature seeds from 18 species of *Habenaria* were collected from the naturally dehiscing capsules from the field as well as from the germplasm bank at the Departmental Botanical Garden, during the years 2010-2014. Light microscopic studies and photography was carried under compound microscope Olympus DM2000 while microphotographic studies of 17 species were carried out using SEM (JEOL-JSM-Model No.-6360) at the Department of Physics, Shivaji University, Kolhapur.

Qualitative as well as quantitative data concerning the general morphology of the seed, i.e. seed surface characters such as size, shape, colour, visibility of embryo, structure of testa, curvature and ridges, ornamentation of periclinal and anticlinal walls and variation in the length/width ratio of seed were studied with light and scanning electron microscopes. Observations were recorded in the observation table. As the various morphometric parameters are strongly variable in a single species, a large number of seeds have been observed in order to get significant means. Orchid embryos are elliptical in cross section, therefore their volume was calculated by using the formula $4/3\ \pi ab^2$, where a = ½ their length and b= ½ their width. Terminologies regarding seed shape and other finer details in SEM used here are those suggested by Arditti *et al.* (1980).

Results and Discussion

Seed Colour (Table 1 and Plate I)

Seed colour ranges from off-white, pale yellow to yellow and pale brown to dark brown. Testa cells are usually transparent, with smooth or reticulated outer cell walls. Pale yellow to yellow coloured seeds were observed in *Habenaria commelinifolia* (Roxburgh) N.Wallich ex Lindley, *H. diphylla* (Nimmo) Dalzell, *H. heyneana* Lindley, *H. longicorniculata* J.Graham, *H. longicornu* Lindley and *H. ovalifolia* R.Wight (Plate I. b, e, j, k, l and n); off-white coloured seeds were observed in *H. digitata* Lindley, *H. foliosa* var. *foetida* (Blatt.

	Name of the species	Colour	Length (L) (mm)	Width (W) (mm)
1	*H. brachyphylla*	Dark brown	0.39 ± 0.16	0.11 ± 0.08
2	*H. commelinifolia*	Faint yellow	0.75 ± 0.22	0.08 ± 0.02
3	*H. crinifera*	Brown	0.47 ± 0.16	0.08 ± 0.03
4	*H. digitata*	Off White	1.69 ± 0.35	0.11 ± 0.05
5	*H. diphylla*	Faint yellow	0.58 ± 0.24	0.33 ± 0.02
6	*H. foetida*	Off White	1.44 ± 0.34	0.11 ± 0.04
7	*H. furcifera*	Off White	1.44 ± 0.54	0.14 ± 0.05
8	*H. gibsonii*	Off White	1.47 ± 0.28	0.09 ± 0.03
9	*H. grandifloriformis*	Dark brown	0.29 ± 0.14	0.16 ± 0.04
10	*H. heyneana*	Yellow	0.54 ± 0.28	0.14 ± 0.06
11	*H. longicorniculata*	Yellow	0.53 ± 0.32	0.14 ± 0.05
12	*H. longicornu*	Faint Yellow	0.67 ± 0.31	0.14 ± 0.04
13	*H. marginata*	Brown	0.47 ± 0.21	0.11 ± 0.02
14	*H. ovalifolia*	Faint yellow	0.97 ± 0.46	0.11 ± 0.06
15	*H. plantaginea*	Off White	0.72 ± 0.40	0.17 ± 0.05
16	*H. rariflora*	Dark brown	0.78 ± 0.35	0.11 ± 0.04
17	*H. roxburghii*	Dark brown	0.61 ± 0.24	0.22 ± 0.08
18	*H. suaveolens*	Dark brown	0.47 ± 0.21	0.14 ± 0.06

Table 1: quantative and qualitative characters

(mean ± standard

& McCann) Bennet, *H. furcifera* Lindley, *H. gibsonii* J.D.Hooker and *H. plantaginea* Lindley (Plate I. d, f, g, h and o), whereas the seeds of *H. crinifera* Lindley, *H. grandifloriformis* Blatter & McCann, *H. marginata* Colebrooke, *H. rariflora* A.Richard, *H. roxburghii* D.H.Nicolson and *H. suaveolens* Dalzell show brown to dark brown colour (Plate I. c, i, m, p, q and r). The colour of the embryo varies from pale yellow to yellow and brown to dark brown. Pale yellow or golden yellow embryos were observed in *H. commelinifolia*, *H. foetida*, *H. furcifera*, *H. gibsonii*, *H. ovalifolia* and *H. plantaginea* (Plate I. b, f, g, h, n and o) while brown to dark brown embryos were observed in *H. brachyphylla*, *H. crinifera*, *H. digitata*, *H. diphylla*, *H. grandifloriformis*, *H. heyneana*, *H. longicorniculata*, *H. longicornu*, *H. marginata*, *H. rariflora* and *H. suaveolens* (Plate I. a, c-e, i-m and p-r).

Length/ Width (ratio L/W)	Number of testa cells	Length of Testa cells (µm)	Width of Testa cells (µm)	Volume of Seed ($mm^3 X 10^{-3}$)
3.50 ± 1.06	4.1 ± 0.56	352.78 ± 55.55	37.50 ± 4.39	1.26 ± 0.50
9.00 ± 1.23	05 ± 0.66	394.44 ± 86.66	40.28 ± 4.94	1.36 ± 0.62
5.67 ± 2.56	5.1 ± 0.73	236.11 ± 60.36	35.83 ± 5.47	0.86 ± 0.12
15.25 ± 3.54	7.9 ± 0.56	520.68 ± 164.28	40.26 ± 4.21	5.48 ± 0.95
1.75 ± 0.69	----	----	----	16.97 ± 2.14
13.00 ± 2.56	08 ± 0.47	463.89 ± 57.15	30 ± 6.11	4.67 ± 0.74
10.40 ± 2.69	5.8 ± 0.41	425.00 ± 72.96	33.89 ± 3.88	7.29 ± 1.49
17.67 ± 3.84	07 ± 0.47	490.26 ± 106.56	36.58 ± 3.54	2.68 ± 0.86
1.82 ± 0.54	3.2 ± 0.42	121.00 ± 10.82	35.00± 5.11	1.85 ± 0.74
3.86 ± 1.25	05 ± 0.66	312.00 ± 71.30	21.06 ± 1.32	2.77 ± 0.95
3.80 ± 1.65	5.2 ± 0.42	261.11 ± 104.09	41.67 ± 4.90	2.66 ± 0.80
4.80 ± 1.36	5.1 ± 0.31	452.78 ± 113.44	40.00 ± 5.27	3.37 ± 0.94
4.25 ± 2.11	4.2 ± 0.42	258.33 ± 91.70	16.39 ± 3.33	1.53 ± 0.85
8.75 ± 2.25	5.8 ± 0.42	488 ± 108.13	36.11 ± 3.70	3.14 ± 0.86
4.33 ± 1.23	06 ± 0.47	336.11 ± 106.74	30.83 ± 4.03	5.25 ± 1.06
7.00 ± 1.68	5.2 ± 0.42	319.44 ± 50.54	27.50 ± 4.62	2.51 ± 0.65
2.75 ± 0.098	4.2 ± 0.42	208.33 ± 69.44	63.06 ± 2.94	7.90 ± 1.24
3.40 ± 1.56	4.1 ± 0.32	383.33 ± 92.40	60.83 ± 3.57	2.38 ± 0.56

of seeds of *Habenaria* species

deviation, N = 10)

Colour of seed varies greatly in immature to mature capsules; in immature capsules the seed is whitish with yellow tinges, whereas the seed in mature capsules is more or less dark brown in many of the species studied.

Seed shape (Table 1 and Plate I)

Microscopic studies reveal three basic seed shapes (1) spathulate in *Habenaria brachyphylla*, *H. grandifloriformis*, *H. roxburghii*, *H. suaveolens* and *H. rariflora* (Plate I. a, i and p-r); (2) fusiform in *H. commelinifolia*, *H. crinifera*, *H. heyneana*, *H. longicorniculata*, *H. longicornu*, *H. marginata* and *H. plantaginea* (Plate I. b, c, j-m and o) and (3) filiform in *H. digitata*, *H. foetida*, *H. furcifera*, *H. gibsonii* and *H. ovalifolia* (Plate I. d, f-h and n). Centrally located oval to elliptical embryos are visible in all species.

Plate I: Seed Diversity in *Habenaria* species

a- *Habenaria brachyphylla*, b- *H. commelinifolia*, c- *H. crinifera*, d- *H. digitata*, e- *H. diphylla*, f- *H. foetida*, g- *H. furcifera*, h- *H. gibsonii*, i- *H. grandifloriformis*, j- *H. heyneana*, k- *H. longicorniculata*, l- *H. longicornu*, m- *H. marginata*, n- *H. ovalifolia*, o- *H. plantaginea*, p- *H. rariflora*, q- *H. roxburghii*, r- *H. suaveolens* (Scale bar = 100 μm)

Seed size (Table 1)
Length of seeds ranges from 0.29 ± 0.14 mm in *Habenaria grandifloriformis* to 1.69 ± 0.35 mm in *H. digitata*. The width ranges between 0.08 ± 0.02 mm in *H. commelinifolia* to 0.33 ± 0.02 mm in *H. diphylla*. Based on the seed length, three distinct groups are formed within the studied species: (1) small (up to 0.7 mm), (2) intermediate (0.7 to 0.9 mm) and (3) large (from 0.9 mm to 2.0 mm) sized seeds. *Habenaria brachyphylla, H. diphylla, H. grandifloriformis, H. roxburghii* (Plate I. a, e, i and q) i.e. the species having dorsiventrally flat leaves on the ground, falls in the category (1). *H. commelinifolia, H. crinifera, H. heyneana, H. longicorniculata, H. longicornu, H. marginata, H. rariflora* and *H. suaveolens* (Plate I. b, c, e, k-n, p and r) come under the category (2). In the category (3), we found *H. digitata, H. foetida, H. furcifera, H. gibsonii* and *H. ovalifolia* (Plate I. d, f-h and n). In the present study the majority of species belonged in the intermediate category. The results are in congruence with the observation made by Molvray & Kores (1995). In *Habenaria*, variations in the seed shape and size can, therefore, be used as an additional taxonomic marker for species identification.

Seed length/width (L/W) ratio (Table 1)
In the present study and according to Arditti *et al.* (1980) and Augustine *et al.* (2001), *H. commelinifolia, H. digitata, H. foetida, H. furcifera, H. gibsonii, H. ovalifolia* and *H. rariflora* were found to have elongate seeds while *H. brachyphylla, H. crinifera, H. diphylla, H. grandifloriformis, H. heyneana, H. longicorniculata, H. longicornu, H. marginata, H. plantaginea, H. roxburghii* and *H. suaveolens* showed truncate seeds. The maximum L/W ratio was observed in *H. gibsonii* (17.67 ± 3.84) whereas the minimum ratio was found in *H. diphylla* (1.75 ± 0.69). Degree of seed truncation can be used as a distinct taxonomic character to differentiate species of *Habenaria*.

Testa cells (Plate II)
During the present investigation it has been observed that the testa cells are transparent, longitudinally oriented, rectangular, elongated with irregular ridges or grooves, whereby the ridges are elevated with a flat and shallow hollow groove in the centre. Cells vary greatly in length and width although their number remains almost constant. The average number of testa cells remained in the range of 4-8 per seed on the longest axis. Molvray & Kores (1995) provided data on the number of testa cells, ranging from 2-20. Vij *et al.* (1992) classified testa cells into three categories

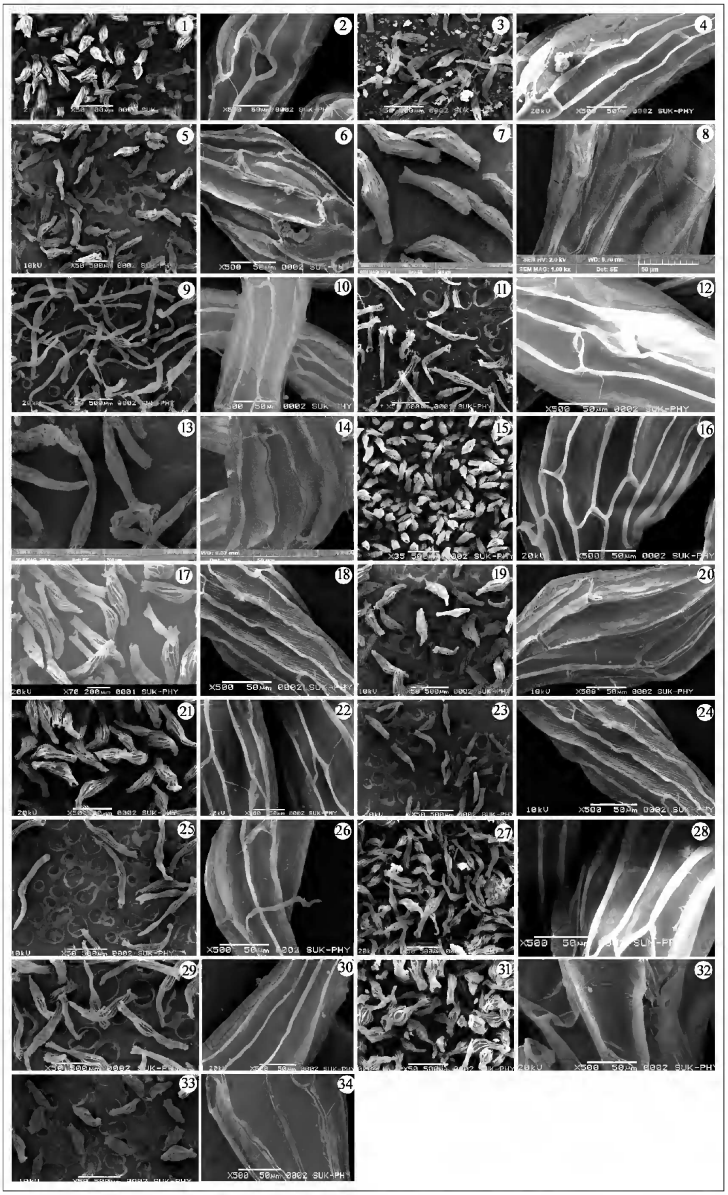

Plate II: SEM of seeds and testa cell structure in *Habenaria* species

H. brachyphylla [1, 2]; *H. commelinifolia* [3, 4]; *H. crinifera* [5, 6]; *H. digitata* [7, 8]; *H. foetida* [9, 10]; *H. furcifera* [11, 12]; *H. gibsonii* [13, 14]; *H. grandifloriformis* [15, 16]; *H. heyneana* [17, 18]; *H. longicorniculata* [19, 20]; *H. longicornu* [21, 22]; *H. marginata* [23, 24]; *H. ovalifolia* [25, 26]; *H. plantaginea* [27, 28]; *H. rariflora* [29, 30]; *H. roxburghii* [31, 32] and *H. suaveolens* [33, 34].

depending upon their size. Cells >200 μm are considered long, 100-200 μm intermediate and cells up to 100 μm short. Our study shows that *H. grandifloriformis* (Plate 2.15 & 16) belongs to the "intermediate" category, whereas the remaining species belong to the "long" category. Maximum length of testa cell was observed in *H. digitata* (520.68 ± 164.28 μm – Plate 2.7 & 8) while minimum length was seen in *H. grandifloriformis* (121 ± 10.82 μm). All other species showed testa cells of intermediate length.

Seed Volume (Table 1)

Arditti *et al.* (1979) proposed a relationship between the volume and the size of the seed. According to them, volume and size are directly proportional to each other. While working on the genus *Bulbophyllum*, Augustine *et al.* (2001) found that higher seed volume is due to greater width rather than length of the testa. In the present investigation similar observations were made. Maximum seed volume has been observed in *H. diphylla* (16.97 ± 2.14 mm$^3 \times 10^{-3}$) due to increase in width (0.31 ± 0.12 mm) while minimum seed volume was reported in *H. crinifera* whose seeds are the narrowest (0.08 ± 0.03 mm) among all the species studied.

Measurement data of embryo (Table 2)

Variation has been observed with reference to length, width and length/width (L/W) ratio of embryo in all studied species. Length shows variations from 0.17 ± 0.09 mm to 0.34 ± 0.17 mm while width shows 0.06 ± 0.01 mm to 0.19 ± 0.10 mm variations. Maximum length of the embryo is observed in *H. roxburghii* (0.34 ± 0.17 mm) while minimum length is observed in *H. grandifloriformis* (0.17 ± 0.09 mm); as for the width maximum is observed in *H. roxburghii* (0.19 ± 0.10 mm) and minimum in *H. crinifera* (0.06 ± 0.01 mm). L/W ratio varies from 1 (in *H. diphylla*, 1.09 ± 0.09) to 5 (in *H. crinifera*, 5 ± 2.98). The majority of the species show a L/W ratio between 1 and 2. Healey *et al.* (1980) suggested that the size of embryo in orchids tends to be uniform within a genus while the volume of the embryo varies from genus to genus. This work was well supported by Augustine *et al.* (2001) while working on the genus *Bulbophyllum*. Arditti *et al.* (1980) suggested that large variations in seed and embryo volumes and percent air space could exist among different populations of the same species. The present study supports the work done by Healey *et al.* (1980) and Augustine *et al.* (2001). Size of embryo in *Habenaria* tends to be uniform at the genus level while the volume of embryo varies greatly. Augustine *et al.*

	Name of the species	Colour	Length L (mm)	Width W (mm)
1	*H. brachyphylla*	Dark brown	0.28 ± 0.16	0.08 ± 0.02
2	*H. commelinifolia*	Yellow	0.28 ± 0.18	0.08 ± 0.02
3	*H. crinifera*	Dark Brown	0.28 ± 0.14	0.06 ± 0.01
4	*H. digitata*	Dark Brown	0.28 ± 0.13	0.08 ± 0.03
5	*H. diphylla*	Brown	0.33 ± 0.19	0.31 ± 0.12
6	*H. foetida*	Dark yellow	0.31 ± 0.17	0.08 ± 0.04
7	*H. furcifera*	Faint Yellow	0.31 ± 0.16	0.08 ± 0.03
8	*H. gibsonii*	Faint Yellow	0.25 ± 0.14	0.06 ± 0.02
9	*H. grandifloriformis*	Dark brown	0.17 ± 0.09	0.14 ± 0.09
10	*H. heyneana*	Dark brown	0.26 ± 0.12	0.12 ± 0.08
11	*H. longicorniculata*	Dark brown	0.19 ± 0.14	0.11 ± 0.07
12	*H. longicornu*	Faint Brown	0.28 ± 0.16	0.11 ± 0.08
13	*H. marginata*	Dark brown	0.22 ± 0.15	0.08 ± 0.04
14	*H. ovalifolia*	Dark yellow	0.22 ± 0.15	0.08 ± 0.03
15	*H. plantaginea*	Faint yellow	0.25 ± 0.16	0.14 ± 0.09
16	*H. rariflora*	Brown	0.22 ± 0.14	0.08 ± 0.03
17	*H. roxburghii*	Dark brown	0.34 ± 0.17	0.19 ± 0.10
18	*H. suaveolens*	Brown	0.22 ± 0.15	0.11 ± 0.09

Table 2: Embryo diversity
(mean ± standard

(2001) admit that in most cases considerable variation can exist in the orchid seed and embryo volume. The embryo in the seed of orchids generally occupies a very small portion of the seed, but in species of *Bulbophyllum* the embryo is large and occupies a major part of the seed. Similar observations were made in few species of *Habenaria*. The embryo of *H. grandifloriformis*, *H. brachyphylla*, *H. rariflora* and *H. suaveolens* occupies a major portion in the seed resulting in increasing weight of the seeds.

L/W ratio	Seed volume (S) ($mm^3 \times 10^{-3}$)	Embryo volume (E) ($mm^3 \times 10^{-3}$)	S/E ratio	Air space (%)
3.33 ± 2.13	1.26 ± 0.50	1.01 ± 0.26	1.24 ± 0.65	19.67 ± 4.62
3.33 ± 2.16	1.36 ± 0.62	1.01 ± 0.28	1.35 ± 0.86	25.95 ± 6.65
5.00 ± 2.98	0.86 ± 0.12	0.45 ± 0.08	1.91 ± 0.98	47.73 ± 8.52
3.33 ± 2.42	5.48 ± 0.95	1.01 ± 0.30	5.42 ± 2.85	81.56 ±11.23
1.09 ± 0.09	16.97 ± 2.14	16.29 ± 4. 36	1.04 ± 0.24	4.00 ± 2.56
3.67 ± 2.54	4.67 ± 0.74	1.11 ± 0.22	4.20 ± 2.13	76.21 ± 9.59
3.67 ± 2.64	7.29 ± 1.49	1.11 ± 0.26	6.57 ± 2.45	84.77 ± 9.45
4.50 ± 2.78	2.68 ± 0.86	0.40 ± 0.09	6.63 ± 3.45	84.91 ±11.34
1.20 ± 1.06	1.85 ± 0.74	1.83 ± 0.45	1.01 ± 0.19	0.86 ± 0.46
2.17 ± 1.64	2.77 ± 0.95	1.96 ± 0.69	1.41 ± 0.90	29.27 ± 8.36
1.75 ± 1.26	2.66 ± 0.80	1.26 ± 0.31	2.12 ± 1.20	52.86 ± 6.53
2.50 ± 1.84	3.37 ± 0.94	1.79 ± 0.40	1.88 ± 1.56	46.68 ± 6.24
2.67 ± 1.95	1.53 ± 0.85	0.81 ± 0.16	1.89 ± 1.65	47.08 ± 8.95
2.67 ± 1.90	3.14 ± 0.86	0.81 ± 0.20	3.89 ± 1.59	74.29 ±11.23
1.80 ± 1.32	5.25 ± 1.06	2.52 ± 0.89	2.08 ± 1.24	51.94 ± 8.26
2.67 ± 1.87	2.51 ± 0.65	0.81 ± 0.22	3.11 ± 1.69	67.87 ± 6.35
1.71 ± 1.20	7.90 ± 1.24	6.60 ± 2.12	1.20 ± 1.02	16.50 ± 4.62
2.00 ± 1.56	2.38 ± 0.56	1.44 ± 0.67	1.66 ± 1.13	39.78 ± 6.52

in seeds of *Habenaria* species

deviation, N = 10)

Seed volume to embryo volume ratio (Table 2)

Earlier studies (Healey *et al.*, 1980; Arditti *et al.*, 1980; Vij *et al.*, 1992; Arditti & Ghani, 2000; Augustine *et al.*, 2001; Swamy *et al.*, 2004; Verma *et al.*, 2013; 2014) showed that higher seed volume is the result of an increase in length and width of seeds and embryos. We found that variations do exist in seed and embryo volumes of all the species studied. Seeds of all the species investigated contain a small, elliptical to oval embryo, within a loosely arranged transparent testa containing air space. There are vast variations in

the volume of seed as well as embryo. The seed volume ($mm^3 \times 10^{-3}$) ranges from 0.86 ± 0.12 (*H. crinifera*) to 16.97 ± 2.14 (*H. diphylla*) and embryo volume ($mm^3 \times 10^{-3}$) ranges from 0.4 ± 0.09 in *H. gibsonii* to 16.29 ± 4.36 in *H. diphylla*. Increase in embryo volume results in increase of seed weight and decrease in air space, while increase in seed volume and decrease in embryo volume increase percent air space which, in turn, results in less weight of seed. In present analysis the ratio of seed volume/embryo volume was found to be highest in *H. gibsonii* and *H. furcifera* (6.63 and 6.57 respectively), leading to wide distribution throughout India. On the contrary *H. roxburghii* and *H. brachyphylla*, with a limited distribution, show lowest S/E volume ratio (1.2 ± 1.02 and 1.24 ± 0.65 respectively).

Air space (Table 2)

Arditti *et al.* (1980), Arditti & Ghani (2000) and Augustine *et al.* (2001) have discussed the significance of the presence of air space in the seeds of orchids, which makes the seeds very light and buoyant. Arditti & Ghani (2000) suggested that increase in the percentage of air space is due to increase in cell length of the testa. In the present work, seeds with maximum percentage of air space were noticed in *H. gibsonii* (84.91 ± 11.34%) followed by *H. furcifera* (84.77 ± 9.45%) whereas seeds with a minimum of air space were observed in *H. grandifloriformis* (0.86 ± 0.46%).

Conclusion

During the present morphometric studies on seeds of *Habenaria* it has been confirmed that various characters studied are of diagnostic value and can be used for taxonomical differentiation between various species as well as for the phytogeographical studies of orchid species.

L/W ratio implies seed's truncation pattern in seven species while remaining are elongated. As length of seed, ratio of seed and embryo volumes increase, percent air space increases which enhances seed buoyancy and long distal dispersal of the species. Seed truncation and testa cell pattern prove to be good morphological characters for distinguishing different species.

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